G-330

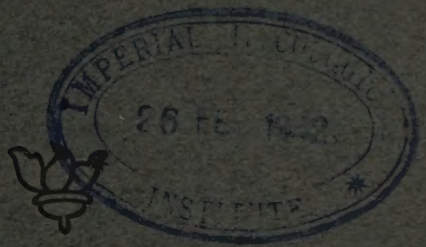
EFFECT OF THE ORANGE-RUSTS OF RUBUS ON THE DEVELOPMENT AND DISTRIBUTION OF STOMATA

BY

B. O. DODGE

(Contribution from Bureau of Plant Industry)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XXV, No. 12 : : Washington, D. C., September 22, 1923



PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1923

EFFECT OF THE ORANGE-RUSTS OF RUBUS ON THE DEVELOPMENT AND DISTRIBUTION OF STOMATA[1]

By B. O. DODGE

Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

In order to obtain a supply of teleutospores with which to carry on some experiments on infecting the black raspberry (*Rubus occidentalis*) with the gametophytic stage of Gymnoconia, the writer has frequently sowed aecidiospores when the topmost leaves of new canes were still folded, while the lower leaves and all those on old canes were fully expanded. Although many trials, both in the field and in the greenhouse, have resulted in failure to obtain infection, the most abundant production of teleutospores followed inoculation of leaves of the latter type. It has been shown repeatedly that aecidiospore germ tubes gain entrance into a leaf through the stomatal openings. Clinton[2] reports that the stomata of leaves of blackberries and raspberries are confined to the lower surface except for a few which occur along the margin on the upper side. He believes, therefore, that infection by aecidiospores of the orange-rust must occur at a time when the side bearing the stomata is exposed to receive the spores falling from above, which would be when the leaves are beginning to unfold. The ventral surface is then turned outward and somewhat upward, and the halves are so folded together as to expose the margins perfectly to catch the spores. As the results of our experiments did not strongly support the theory that leaves are the most readily infected before they unfold completely, a study was made of the development and distribution of stomata on various types of leaves from blackberries and raspberries. The fact that agitation by the wind and the work of insects might well carry spores to the underside of fully expanded leaves would not minimize the importance of the distribution of stomata in facilitating infection. In the course of this work it was found that the invasion of leaves by the orange-rust mycelium has a very unusual effect on the production of stomata.

If account is taken of the conditions of the leaves of our blackberry or raspberry in nature at the time aecidiospores are being shed, it will be seen that several types are exposed to infection. The first leaves to unfold are the ones on the normal old canes; the infected old canes are also developing their leaves at this time. Practically all leaves on the old canes, normal and infected, are of about the same age. New shoots from the base of infected and of uninfected plants grow up a little later. During the growth period of the new canes there will always be leaves just unfolding at the top, and others lower down fully expanded.

In general, most of the stomata on normal leaves of Rubus are on the lower side, as stated by Clinton. A number of species of this genus have a few on the upper side at the tips of the serrations. Some blackberry leaves examined have a small number irregularly scattered, singly or in groups of two or three, on the upper side, especially along the larger

[1] Accepted for publication June 25, 1923.

[2] CLINTON, G. P. ORANGE RUST OF RASPBERRY AND BLACKBERRY. Ill. Agr. Exp. Sta. Bul. 29, p. 273-300, 4 pl. 1893. Literature, p. 292-295.

Journal of Agricultural Research, Washington, D. C.
agn.

Vol. XXV, No. 12
Sept. 22, 1923
Key No. G-330

69982—23

veins; in every such case the number is so small as to be of little consequence in the economy of the normal plant. It is quite otherwise in case of plants systemically infected with either of the orange-rusts.

Sections of leaves collected at random for another purpose from a systemically infected blackberry in New Hampshire show fully twice as many stomata on the upper side as they do on the lower side. An examination of sections of black raspberry leaves which had been prepared for a study of young aecidia revealed an abundance of stomata on the upper side. Further investigations disclosed the fact that the systemic stage of these orange rusts regularly so affects the host as to lead to the development of a large number of stomata on the upper side of the leaf where normally there would have been only a very few or none at all. One frequently sees that in a blackberry which has harbored the rust for two years or more most of the leaves on the old canes infected are evenly covered with spermogonia from the time they begin to unfold. From one-fourth to one-half of the total number of stomata on such leaves will be found on the upper side. Certain specimens of infected mountain blackberry did not show such a large percentage of stomata on the dorsal side; the increase in the number due to the stimulus of the rust was very marked. The development of spermogonia on leaves of primarily infected blackberries, to be noted later, is frequently inhibited, aecidia alone being formed. Neverthless, by noting the areas where stomata occur in large numbers on the upper side one can tell several days in advance just where aecidia are to be developed. Whenever the rust at maturity covers only a part of the leaf it will be found that only that part is provided with additional stomata on the upper side. As long as the epidermis is in a plastic condition the advance of the mycelium into new areas is accompanied by the development of additional stomata.

The effect of the parasite on the production of stomata varies with the course of development of the rust. Under certain conditions, spermogonia may be distributed uniformly over the leaf from the first, but the formation of aecidia is long delayed or is entirely omitted. There will be about the same number of stomata on both sides of the leaf, the normal number occurring on the under side. It has also been found that when certain blackberries show the rust for the first time after primary infection by sowing sporidia of the short-cycled rust, the spermogonial stage is entirely lacking. The infected leaves show by their yellowish-green margins the extent to which the rust hyphae have penetrated. Aecidia will later develop along the discolored margins, then gradually spread toward the midrib, preceded by the fading out of the natural green and the production of stomata on the upper side. Very few spermogonia were found on some 50 plants of the varieties Kittatinny and Iceberg the first two years following infection; aecidia developed normally. Other varieties, Ancient Briton, Blowers, etc., developed spermogonia under similar conditions. The complete suppression of this stage in the life cycle of a rust is said to be of rare occurrence. A further study of the rust on different varieties of blackberry may serve to suggest a reason for the incomplete development or nonproduction of spermogonia by other rusts such as the one on the mallows.

In rare cases aecidia also fail to appear, most of the leaf merely becoming yellowish. Such leaves, though never bearing any spore forms, have been invaded by mycelium, as shown by the large number of stomata on the discolored areas of the upper side, the usual number appearing below.

The gametophytic mycelium of Gymnoconia does not always penetrate into every part of the leaf of, for example, the black raspberry. One-half only may be infected; variegations occur or angular patterns are laid out, the space between certain large veins escaping attack. Photographic prints can be made directly from these leaves, either before or after the chlorophyll has been extracted (Pl. 1). It will be found that production of stomata on the upper side of the leaf is coincident with the invasion of an area by the orange-rust hyphae. Plate 1, C, shows that the infected area of the raspberry leaf is sharply limited on the left side by the vein, and toward the tip on the right side the mycelium has spread out part way between the second and third veins. There were no stomata on the dorsal side of this leaf, except over the infected areas. A somewhat different pattern was worked out in the leaf shown as Plate 1, A. Dorsal stomata occurred on the left side except at the base, into which

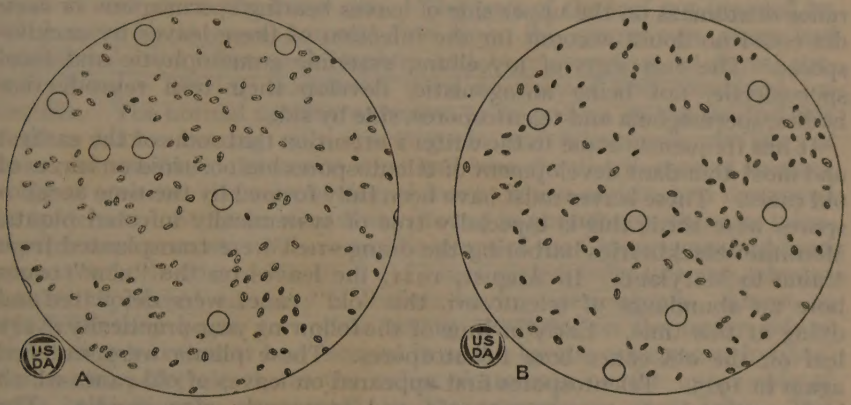


FIG. 1.—Relative numbers of stomata on corresponding areas from the ventral and dorsal sides of a leaf from an old cane of *Rubus occidentalis* infected with the long-cycled orange-rust. Position of stomata and spermogonia determined with the aid of a camera lucida: size and shape of stomata purely diagrammatic. In fact, the stomata on the dorsal side are on the average somewhat larger. Circles represent positions of spermogonia. The fields diagramed are such as were covered by a 16 mm. lens and Leitz Periplane 10x ocular. A, Area from ventral surface; B, Similar area from the dorsal side nearly opposite the area shown in A. There were 150 stomata in the area from the ventral side, and 136 in the dorsal area opposite. It would be necessary to examine several such fields before a single stoma would be found on the dorsal side of an uninfected leaf, or of an uninfected part of this same leaf.

hyphae had not penetrated. The lighter areas on the terminal and left lateral leaflets (Pl. 1, B), represent portions invaded and where dorsal stomata were numerous. The leaflets which escaped infection had no stomata on the upper side. Plate 1, F and G, shows prints of leaflets of Kittatinny blackberry on parts of which aecidia were just maturing. Practically the same number of stomata was found on the upper and lower sides on areas infected.

The time intervening between the opening of a particular infected leaf on new canes and the maturing of aecidia on that leaf becomes progressively shorter as the season advances, until a time is reached when fully formed aecidia are exposed as the leaf opens. What should be the effect of such an early appearance of aecidia on the underside of the leaf? The development of stomata on the underside is, just as we might expect, greatly interfered with, there being only a few normal stomata on that side, and these are at the tips of the serrations upon which no aecidia appear, just the reverse of the distribution of stomata in the normal leaf. An accurate count of the stomata has not been made on any considerable number of areas of normal and of infected leaves, but it is certainly inter-

esting to note that soon after the period is reached in a vigorous blackberry shoot when mature aecidia appear with the unfolding of the leaf, the tip of the new cane begins to outstrip the parasite in the upward growth, and the production of aecidia ceases; the grower says, "the plant is recovering."

That it is the gametophytic mycelium and not the sporophytic that has the power of inducing this morphological change in the host leaf is clear from a comparison of two sets of leaves, each bearing teleutosori. One collection was made July 10 at Lakewood, N. H. The leaves were thickly covered with spermogonia accompanied by an abundance of teleutosori, the aecidial stage having been suppressed. There were about the same number of stomata on both sides of the leaf. The other collection was made September 6 in Maine; these leaves, of course, bore only teleutospores. There were no stomata on the upper side, while the lower side was provided with what seemed to be the normal number. The occurrence of stomata on the upper side of leaves bearing spermogonia or aecidia could no doubt account for the infection of these leaves by aecidiospores. The two sorts of mycelium, systemic gametophytic and local sporophytic, not being antagonistic, develop their own reproductive bodies, spermogonia and teleutospores, side by side.

It has frequently come to the writer's attention that some of the earliest and most abundant development of teleutospores has occurred on leaves of old canes. These leaves must have been fully formed by the time aecidiospores were shed; this is especially true of systemically infected plants. Mountain blackberries harboring the orange-rust were transplanted from Maine to Maryland. In August, 1921, the leaves on the "new" canes bore an abundance of teleutosori, the "old" canes were defoliated and dying at this time. Early in June of the following year practically every leaf on the old canes bore teleutospores. These plants were watched again in 1923. Teleutospores first appeared on leaves of old canes which had previously borne spermogonia and frequently also aecidia. The position of the leaf on the cane, whether at the tip or at the base, was clearly of no great importance. The leaves on the new canes remained free from the telial stage during the summer of 1922. All leaves on the old canes had unfolded at about the same time and had borne aecidia, and would have been equally susceptible so far as maturity is concerned. On the other hand, leaves develop on the new canes one by one, as previously noted, so that as aecidiospores are shed leaves of different ages would be exposed. In this particular case in 1922 either the leaves on the new canes had not opened or they were not sufficiently mature when the spores were shed.

After seeing that leaves harboring the gametophytic mycelium were provided with additional stomata on the upper side, it occurred to the writer that this might account for the production of telia on so many leaves of the old infected canes. Aecidiospores were sowed on the upper side of leaves bearing spermogonia, the leaves then being placed in damp chambers. After two days the leaves were dropped in Flemming's fixture. The germ tube grows along the surface until it comes into the immediate vicinity of a stoma, then, if necessary, the end turns sharply, broadens out and sends the infection tube through the opening. The method of penetration was observed without sectioning by removing the chlorophyll from leaves killed in the Flemming's fluid. There were in the greenhouse on April 18 a number of potted plants of *Rubus occidentalis* systemically infected with the Gymnoconia, and now showing spermogonia. On many leaflets, especially on old canes, spermogonia

occurred only on a part of the leaf (Pl. 1, A). Aecidiospores were sowed over several plants in such a way that most of the spores fell on the dorsal side of leaves which had fully expanded. Many spores must have come in contact with the underside. The leaves on the tips of young basal shoots which had not unfolded were tagged. Particularly large numbers of spores were sowed on these folded leaves. The experiment was repeated April 25 with other plants. On May 25 teleutosori in abundance were found on plants from both sowings, showing that it takes at least four or five weeks in the greenhouse for the teleutosporic stage to reach maturity. Some of the leaves now bearing teleutosori also bore aecidia and spermogonia, while others bore only spermogonia; teleutospores were more abundant on such leaves.

In case aecidia or spermogonia occurred on only a part of a leaf, it was found that teleutosori were present only on that part, often on the upper as well as on the lower side. The part of the leaf not invaded by the gametophytic mycelium and free from the orange-rust stage had escaped infection when aecidiospores had been sowed on its dorsal surface. Leaves bearing teleutosori were decolorized and examined for stomata. The normal number appeared to be present on the ventral side of those leaves bearing only spermogonia and teleutosori. It was, of course, impossible to determine what had been the distribution of stomata on those areas on the ventral side of leaves where aecidia had destroyed the epidermis. Stomata were always found in abundance wherever teleutospores were present. Where aecidiospores of the Gymnoconia had been sprayed on both sides of leaves of old canes systemically infected, teleutosori first appeared on parts of leaflets having spermogonia. About 10 days later they began to appear among the hairs on the under side of neighboring leaves that had escaped invasion by the orange-rust hyphae. The results point clearly to the value of stomata on the upper side of leaves in facilitating the attack by the sporophytic germ tube. The gametophytic mycelium of the rust stimulates the host to provide ready means of access by the sporophytic stage which is to follow later.

As previously noted, the leaves of the black raspberry which are exposed to infection as the aecidiospores are being shed, may be grouped as follows:

1. Fully expanded but somewhat dwarfed leaves on old canes already infected with the systemic or orange-rust stage. The leaves or parts of leaves into which the gametophytic hyphae have penetrated will be rather devoid of hairs on the lower surface and have an abundance of stomata on the dorsal side, two factors favoring infection by the germ tubes.
2. Large, fully unfolded leaves on infected basal shoots from systemically infected plants such as produce the old canes in No. 1. Gametophytic hyphae evenly distributed throughout most of the leaflets, which will later usually be covered with aecidia. Very few hairs on the lower side, many stomata on the dorsal side.
3. Fully expanded leaves on normal old canes. Leaves somewhat tomentose on the underside. No stomata on the dorsal side (a very few at the tips of the serrations).
4. Very young leaves at the tips of systemically infected basal shoots. Some of these leaves will be tightly folded, others just expanding.
5. Fully expanded and growing leaves, tomentose beneath, on normal basal shoots.
6. Very young leaves, some still folded and very tomentose, others expanding, on basal shoots of normal canes.

The infection experiments described above show that some of the factors which determine the readiness with which a leaf of the raspberry can be infected by sowing aecidiospores resulting in the development of the teleutosori (*Puccinia peckiana*) are: Presence or absence of stomata

on the dorsal side, the maturity of the leaf, and the amount of tomentum covering the stomata. Leaves such as would come under groups 1 to 3 above are certainly most easily infected, and in the order given. Owing to the fact that it takes teleutosori a much longer time to mature on leaves of normal new canes, one is apt to be misled as to the relative susceptibility of the very youngest leaves. Very few teleutospores were ever developed on leaves which were still folded when the sowings of aecidiospores were made, even though these leaves were systemically infected, and therefore devoid of tomentum. The same line of infection experiments has also been carried on with blackberries and dewberries, with the same results. Teleutospores appear first and most abundantly if aecidiospores are sowed on leaves of the old canes that are systemically infected. The comparatively short time (four or five weeks) required for the development of teleutosori on leaves already harboring the orange-rust stage is accounted for by the rapidity with which such leaves reach maturity and fall off. The ease with which they may be infected is at least in part due to accessory dorsal stomata and freedom from tomentum.

Blodgett [3] reported that he had observed that blackberries bearing orange rust wilted sooner than uninfected plants, and that this was due to excessive transpiration.

Reed and Crabill [4] think that the greater loss of water by the rusted plant reported by Blodgett was due to the rupture of extensive areas of the ventral epidermis, which would facilitate evaporation. "Possibly other factors connected with the diseased condition may also operate to cause increased transpiration." These authors found that leaves of apples affected with Gymnosporangium transpire about the same whether they are in daylight or darkness. Healthy leaves transpire much more rapidly in daylight. The average for daylight and darkness, however, is practically the same for diseased and for healthy leaves. They think that the parasite must in some way affect the operation of the stomata. The substomatal cavities of rusted apple leaves are obliterated. Their figure 13 shows stomata only in the ventral epidermis of an area bearing spermogonia and aecidia.

In an isolated plant infected with Gymnoconia the chances for the production of telia are certainly vastly increased should the leaves which will some day have their ventral surface covered with blister-like aecidia be provided with stomata on the other side. Just this occurs as, we might say, a matter of safety, if not always of necessity, when leaves are systemically infected. Aecidia frequently fail to develop in leaves covered with spermogonia. In such a case there is no apparent reason why stomata on the ventral surface should not function properly. The accessory dorsal stomata are formed as the result of the stimulus of the gametophytic mycelium present just beneath the epidermis. The failure to produce aecidia following spermogonia is undoubtedly due to the condition of the host leaf, but it is not a condition under the control of the host. A great many investigations of the development and distribution of stomata have been made in the past, but the writer has been unable to find in the literature an account of another such curious interaction between host and parasite. There are several systemic rusts known which attack herbaceous plants. It would be interesting to know whether additional dorsal stomata are also developed by these hosts.

[3] BLODGETT, FREDERICK H. TRANSPIRATION OF RUST-INFESTED RUBUS. *In* Torreya, v. 1, p. 34–35. 1901.
[4] REED, HOWARD S., and CRABILL, C. H. THE CEDAR RUST OF APPLES CAUSED BY GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE SCHW. Va. Agr. Exp. Sta. Tech. Bul. 9, 106 p., 23 fig. 1915. Bibliography, p. 104–106.

PLATE 1

The prints were made by bringing the leaves, from which the chlorophyll had been removed, in direct contact with Velox paper.

A.—Black raspberry leaflet, dorsal side. One-half of the leaflet, except a small portion of the lower left, infected and bearing spermogonia. Stomata on the upper side only where hyphae are present.

B.—Leaf of black raspberry, ventral surface. Left half of terminal leaflet infected; hyphae are beginning to invade the right half. Most of the left basal leaflet is infected; dark area along the midrib devoid of hyphae. No stomata were found on the dorsal side of the right basal leaflet, which was not infected, and none on the upper side of the others except where infected.

C.—Black raspberry, ventral surface. At the left the boundary line between infected and uninfected areas is very clear cut, being limited by a large lateral vein. On the right side the mycelium is advancing from the margin into the regions between the second and third veins; stomata present on the dorsal side only where leaf is infected.

D and E.—Leaflets from the same plant one month later; that is, one month after sowing aecidiospores of the Gymnoconia on the upper side of these leaves. Dorsal stomata and teleutosori only on areas now bearing aecidia. Teleutosori also on ventral side among aecidia.

F.—Leaf of Kittatinny blackberry aecidia maturing. About the same number of stomata were found on both sides of the portions of the leaflets bearing aecidia. No stomata on dorsal side of basal leaflet at the right.

G.—Larger leaflet of Kittatinny blackberry, showing that the fungus advances from the margin toward the midrib. Dorsal stomata only on the area where aecidia are present on the ventral side. No spermogonia were formed.

○

A
B
C
D
E
F
G
USDA